A FLUORESCENT PCR ASSAY FOR THE CODOMINANT INTERPRETATION OF A DOMINANT SCAR MARKER

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Introduction

A major disadvantage of many PCR based markers is that they exhibit dominant inheritance and cannot be used to discriminate between homozygous (AA) and heterozygous (Aa) genotypes. Codominant markers provide greater efficiency than dominant markers for purposes of marker assisted selection (see review by Kelly and Miklas, 1998).

	AA	Aa	aa
Dominant marker	———	———	
Codominant marker	———	———	
		———	———

The SCAR marker $SBD5_{1300}$ (Miklas et al., 2000) is a dominant marker that is tightly linked to the *bc-1*^{2} allele conditioning resistance in common bean to Bean Common Mosaic Necrosis Virus (BCMNV). An F_2 population segregating for the *bc-1*^{2} gene was used to investigate whether codominant interpretation of a dominant SCAR marker was possible using a real-time fluorescent PCR assay specific for $SBD5_{1300}$ (Vandemark and Miklas, 2002).

Materials and methods

Plant materials. The segregating population consisted of 59 F_2 plants derived from a cross between pinto bean lines P94207-43 (*bc-1*^{2}*//bc-1*^{2}) and P94207-189 (*bc-1//bc-1*) (Miklas et al., 2000).

PCR primer and probe design. The 1329 bp DNA sequence corresponding to the $SBD5_{1300}$ SCAR marker was analyzed using Primer Express software (Applied Biosystems, Foster City, CA).

Forward primer p43335F: 5´-TGTACTGTGCTACCACTGCTACATCTT-3´
Reverse primer p43424R: 5´-6FAM-CAGAGCTCAGAATTGCAGCAA-TAMRA-3´.
Taqman Probe p43T369C: 5´-ATGCTCCCTCACATTC ATTTAAGTTTGCTGCATAT-3´.

The primer/probe set amplified a 90bp fragment.

TaqMan™ assays. PCR for each sample was performed in 50 µl reactions containing100 ng of purified genomic DNA, 900 nM forward primer p43335F, 900 nM reverse primer p43424R, 100 nM TaqMan™ probe p43T369C, 5 µl ddH_2O, and 25 µl of 2X TaqMan™ Universal PCR Master Mix (Applied Biosystems). Amplifications and detection of fluorescence were done using a GeneAmp 5700 Sequence Detection System (Applied Biosystems). The thermocycling profile for all PCR reactions was the manufacturer's suggested default cycling profile.

Genotyping Plants with Quantitative PCR.

1. The relative amount of *bc-1*^{2} present in 100 ng DNA for each plant was determined by plotting the C_T value for the PCR reaction on the standard curve plot generated using DNA of the homozygous dominant *(bc-1*^{2}*//bc-1*^{2}*)* parent P94207-43 (Figure 1).

2. Four F_1 plants, determined to be heterozygous (*bc-1*^{2}*//bc-1*) based on the results of progeny tests were used as a comparative reference sample for genotyping segregating F_2 plants.

3. A group mean (y) and standard deviation (s_y) was calculated for the comparative reference sample based on the combined analysis of three PCR reactions for each plant. A 99% confidence interval for all heterozygotes was determined using the formula $y \pm 2.58s_y$.

4. Genotype of F_2 plants were assigned as follows based on the mean of four replicate PCR reactions per plant:

A. F_2 plants that fell within the confidence interval were classified as heterozygotes (*bc-1*^{2}*//bc-1*).

B. F_2 plants which fell outside the tail area to the right of the confidence interval were classified as homozygous dominant (*bc-1*^{2}*//bc-1*^{2}).

C. F2 plants with no fluorescence were classified as homozygous susceptible (bc-1//bc-1).

Real time amplification profiles for the three genotype are depicted in Figure 2.

Figure 1. Standard curve using known quantities (7.82, 15.63, 31.25, 62.5, 125, and 250 ng) of purified genomic DNA of the homozygous dominant (*bc-1*^{2}*//bc-1*^{2}) parent P94207-43.

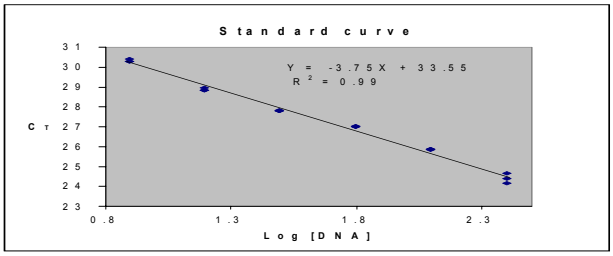
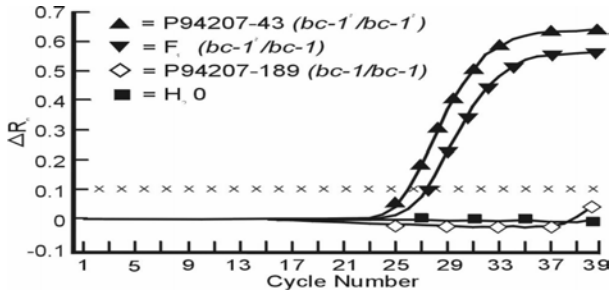


Figure 2. Real-time amplification plot of total DNA isolated from bean plants having different genotypes for the *bc-1*^{2} allele. For each reaction, relative fluorescence (ΔR_N) is plotted versus the number of PCR cycles.

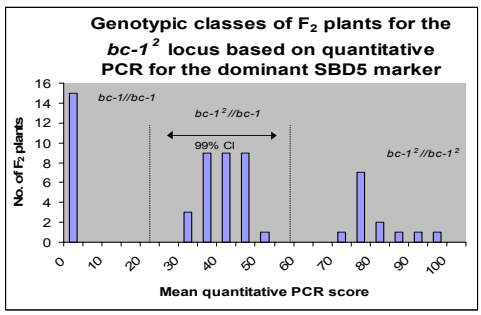


Results

1. The allelic state of 59 F_2 plants for the *bc-1*^{2} allele was confirmed by F_3 progeny tests for reaction to NL-3 strain of BCMNV.

2. The real-time PCR assay for the dominant $SBD5_{1300}$ SCAR marker genotyped the F_2 plants for the *bc-1*^{2} allele with 100% accuracy when genotyping was based on mean quantitative PCR results relative to a 99% heterozygote confidence interval (Figure 3). The assay was 98% accurate (58/59) when genotype assignment was based on a 95% heterozygote confidence interval.

Figure 3. Frequency histogram for the mean of three quantitative PCR reactions for each of 59 F_2 bean plants derived from the cross P94207-43 (*bc-1*^{2}*/bc-1*^{2}) x P94207-189 (*bc-1//bc-1*). PCR was performed for each plant using 100 ng of total genomic DNA.



Discussion

1. This approach for codominant interpretation of a dominant marker may be broadly applicable to other alleles provided a reference sample of known heterozygotes are available for developing confidence intervals for assigning genotype.
2. Applying this strategy will reduce costs associated with breeding programs because it will not be necessary to produce seed and perform progeny tests to identify homozygotes.
3. Efforts are in progress to develop similar assays for other alleles conditioning disease resistance in bean and to simultaneously genotype plants for multiple alleles by multiplexing real-time fluorescent PCR assays.

References

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Miklas P. N., Larsen R. C., Riley R. and J. D. Kelly. Potential marker-assisted selection for *bc-1*^{2} resistance to bean common mosaic potyvirus in common bean. Euphytica 116:211-219 (2000).

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